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Role of Bioinoculant (AM Fungi) Increasing in Growth, Flavor Content and Yield in *Allium sativum* L. under Field Condition

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Abstract

Present investigation deals with the beneficial effect of Arbuscular Mycorrhiza (AM) fungal species *Glomus fasciculatum* on growth performance of *Allium sativum* under field condition. In AM inoculated garlic plant showed significant increase in plant growth parameters like plant height, total biomass and bulb diameters, bulb weight, and yield. There was 21.10 % increment in yield of *Allium* bulb as compared to non AM inoculated garlic plant. Percentage of AM colonization occurred at all sampling and the colonization was higher during bulb initiation. Mycorrhiza inoculated garlic plant showed more alliin and allinase enzyme activity as compared to non inoculated garlic plant. The results reveal that mycorrhiza contribute to growth and development of garlic plant under field condition.

Keywords: AM fungi, alliin, allinase, garlic, yield

Introduction

AM fungi are belonging to recently describe *Phylum Glomeromycota*. (Redecker *et al.,* 2000; Schußler *et al.,* 2001). Obligate symbiotic association with roots of 90% of all vascular plant. AM fungi improve plant growth profoundly through increased uptake of phosphorus, nitrogen and other nutrients. The use of AM fungi in enhancing plant growth and yield of many crops has gained momentum in recent years because of the higher cost and hazardous effects of heavy doses of chemical fertilizers. The extra-matrical hyphae of AM fungi act as extensions of roots and increase the surface area of the root system, making it more efficient for absorption of water and diffusion-limited nutrients, this effect being more pronounced in P-deficient soils (Bagyaraj and Reddy, 2000). Other beneficial effects are in the biological control of root pathogens, biological nitrogen fixation and increased ability to withstand abiotic stresses. Though benefits of AM fungi under field conditions have been reported for annuals and perennials inoculated in the nursery (Bagyaraj, 1984; Chandrashekara *et al.,* 1995),

Garlic (*Allium sativum* L.) has been known as folk medicine, since ancient time. *Allium sativum* L. is one of the crops growing in one lakh hectar area of arid and semiarid regions of India. Its an important bulb crop widely used as a spice/condiment, medicinally garlic is used against arteriosclerosis, high blood pressure, and has been shown to have antibacterial, antifungal, antiviral and antiprotozoal activities. It also modulates the cardiovascular and immune system and has antioxidative and anticancerogenic properties (Harris *et al.*, 2001).

India is second largest country after China in garlic cultivation, but the productivity will be less than in other countries. *Allium species* including garlic are responsive to AM symbiosis. In absence of AM fungi onion growth is stunted (Smith and Read, 1997; Gerdemann, 1968). *Allium sativum* has been used as a bactericidal medium activity against wide range of organisms. This activity has been attributed to one of its compound allicin produce from alliin by allinase enzyme present in garlic. The characteristic flavor of *Allium* species is caused after the enzyme alliinase hydrolyses the cysteine sulfoxides to form pyruvate, ammonia and Sulfur containing volatiles. Alliinase is extremely abundant in garlic tissue, consisting of at least 10% of the total clove protein (Van Damme *et al.,* 1992). Allicin is produced by enzymatic degradation of alum, S-allyl- L-cysteine-S-oxide. Inhibition of sulfhydryl enzymes is associated with the pres-

Major garlic producing countries (2005)

	Area (1000 ha.)	Production (1000 MT)	Yield (ton/ha.)
China	640.0	110.0	17.19
India	120.0	5.0	4.17
Export of garlic from India			
	Qty (MT)	Value (Rs.Lakh)	
2006-2007	11858.93	1824.39	

ence of the –SO-S-grouping (Wills, 1956). The functional flavor components of Allium plants are organo-sulfur (S) compounds that are synthesized from a common precursor, the S-alk(en)yl cysteine sulfoxides (ACSOs). After disruption the enzyme alliinase acts upon the ACSOs in the cytoplasm, giving rise to the volatile compounds responsible for the characteristic flavor and aroma of the plants (Lancaster and Collin, 1981) with the formation of pyruvic acid and ammonia as by-products.

The purpose of this was study to use the AM fungal species *Glomus fasciculatum* in garlic (*Allium sativum* L.) plants for enhancement of growth, flavor and yield under field conditions.

Materials and methods

Plant material and experimental design:

The cloves of garlic (*Allium sativum* L.) were surface sterilized with 0.01% $HgCl_2$ and field experiment was conducted in Botanical Garden, Department of Botany, University of Pune India. Raised planting beds were prepared. Plot dimensions were 1.5 m x 1.5 m (0.00015 ha) Treatments included mycorrhizal garlic plants inoculated with Glomus fasciculatum and non mycorrhizal garlic plants. Sandy loamy soil, field capacity 0.23 cm^3 water/cm^3 soil. Soil was analyzed as follows: sand 80% silt 15% and clay 5% by website: www.pedosphere.com

Three month old 1-kg. of *Glomus fasciculatum* (GF) species of mycorrhizal inoculum which was grown on *Guania* grass, containing AM colonized roots, rhizosphere soil (extrametrical mycelium and spores) (10 – 15 spores/gm) was taken. The experimental design consisted of total two treatments non mycorrhizal (C) and mycorrhizal (GF) inoculated plot, in each plot 134 cloves were sown. Observations were recorded after 60, 90, 120 days and after harvest of AM inoculated garlic plants.

Morphological parameters:

Number of leaves, leaf area, plant height, bulb diameter, fresh weight, dry weight, percent root colonization, bulb yield, alliin content and allinase activity (Schwimmer and Guadagni, 1962).

AM colonization:

Roots of selected plants were stained for AM colonization (Phillips and Hyaman, 1970). Percentage of AM colonization was estimated by Gridline intersect slide method (Giovanetti and Mosse, 1980).

Statistical analysis:

Data analysis was done by Student t test and the values are mean ±SD for * $p \leq 0.05$ was considered as significant. Ns: non-significant, *: significant

Results and discussion

Under field conditions the mycorrhizal garlic plants showed significant increment in plant growth. After 60, 90 and 120 days of AM inoculation, mycorrhizal garlic plants showed significant increase in leaf number as compared to non-mycorrhizal garlic plants (Tab. 1.). Leaf area and plant height increased significantly after 90 and 120 days of AM inoculation in garlic plants as compared to non-mycorrhizal garlic plants (Tab. 1.). After 60, 90 and 120 days of AM inoculation, mycorrhizal garlic plants showed significant increase in bulb diameter and plant fresh weight as compared to non-mycorrhizal garlic plants (Tab. 1.). Plant dry weight increased significantly as compared to non-mycorrhizal garlic plants after 90 and 120 days of AM inoculation (Tab. 1.). *Allium* spp., including garlic (*Allium sativum* L.), are responsive to AMF symbiosis, and stunting of garlic growth in the absence of AMF has been reported (Smith and Read, 1997; Gerdemann, 1968; Koch *et al.,* 1997). Stunting of garlic bulbs reduces not only the yield of the crop, but also the commercial value of the crop by yielding small sized bulbs (Koch *et al.,* 1997).

As shown in Fig.1., AM inoculated garlic plants showed increased root colonization. After 60 days of AM inoculation it was 28.33%, after 90 days of AM inoculation it was 45%, after 120 days of AM inoculation 61.66% and after harvest 75%. AM fungal inoculation increased the level of AMF root colonization of garlic. This increase in colonization is very important for the growth and nutrient uptake by the plant and the higher number of spores which may compete with native AM spores. It may also indicate that the introduced *Glomus fasciculatum* was more efficient than the native fungi. This suggests the potential for supplementing or improving native AM fungal population to benefit plant growth and hence increase bulb yield.

The alliin content in mycorrhizal garlic plants was 50% higher after 60 days of AM inoculation. The percent incre-

Tab. 1. Morphological parameters in *Allium sativum* L. after 60, 90 and 120 days of AM treatment

Parameters	60 days		90 days		120 days	
	C	GF	C	GF	C	GF
No.of leaves	4.33±0.47	5.66±0.94*	5.33±0.47	6.33±0.47*	7.33±0.47	7.66±0.47*
Leaf area (cm^2)	18.33±1.54	20.83±2.65^{Ns}	26.83±0.84	31.06±1.72*	36.46±2.00	41.7±1.60*
Plant height (cm)	42.53±2.22	45.26±0.88^{Ns}	44.66±2.77	51.73±1.32*	45±2.16	52.33±1.24*
Bulb diameter (cm)	2.43±0.24	3.10±0.48*	4.26±0.20	4.66±0.16*	6.43±0.53	6.8±0.29*
Fresh weight. (g)	2.79±0.56	4.74±1.35*	7.33±0.06	10.34±0.91*	11.8±2.21	17.08±2.24*
Dry weight (g)	0.50±0.21	0.63±0.096^{Ns}	0.67±0.40	1.32±0.07*	1.72±0.32	2.55±0.16*

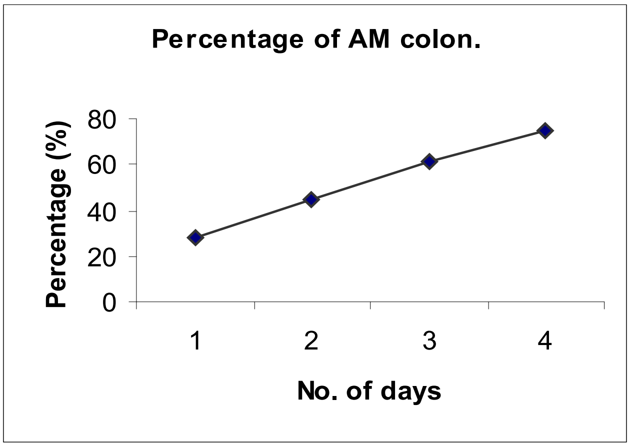

Fig. 1. Percentage AM colonization in *Allium sativum* L. after 60, 90, 120 and 150 days of AM treatment

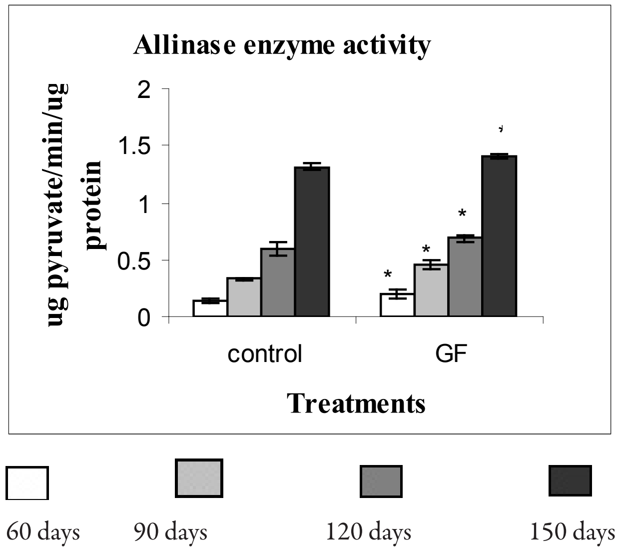

Fig. 3. Allinase activity in *Allium sativum* L. after 60, 90, 120 and 150 days of AM treatment

ment decreased with increase duration i.e. 22% increment after 90 days, 13% after 120 days and up to 4% after 150 days of AM inoculation. Allinase activity was found to be significantly higher in AM inoculated garlic plants as compared to non-AM inoculated garlic plants. The percent increment was 45%, 37%, 15% and 7% higher in AM inoculated garlic plants after 60, 90, 120 and 150 days of AM inoculation respectively (Fig. 2., 3.). Alliinase is the enzyme that initiates the conversion of the alkyl cysteine sulphoxide flavour precursor (alliin) to allicin and its derivatives. Alliinase is extremely abundant in garlic tissue consisting of at least 10% of the total clove protein (Van Damme *et al.,* 1992).

From the above data it was observed that after 60 days i.e. five leaf stage and 90 days i.e. bulb filling stage of AM inoculation, the alliin and allinase activity was found to be higher in AM inoculated garlic plants as compared to non AM inoculated garlic plants under field conditions. During five leaf and bulb filling stage, activity of allinase was found to be higher which resulted in more production of sulpher containing compound i.e. alliin. Bloem *et al.* (2004) reported the translocation of alliin from leaves to bulbs at bulb filling stage in onion. This increase in alliin content gives pungency to garlic plants. Previous experiments showed that mycorrhizal fungi tended to affect the pungency of *Allium cepa* (Guo *et al.,* 2006a) and *Allium fistulosum* (Guo *et al.,* 2006b). The increased absorption surface area offered by the extended soil network of fungal hyphae external to roots (Kothari *et al.,* 1991) might have increased P supply and promoted plant growth to give high bulb yields. Iqbal and Qureshi (1972) reported 85% increase in height of sunflower plants inoculated with AM fungi compared to uninoculated controls under field conditions. Solubilization of unavailable forms of P through soil acidification by root exudates of mycorrhizal plants is also believed to enhance P uptake (Bolan *et al.,* 1984). Since P is relatively immobile in soil and transfer mainly occurs by diffusion to the root surfaces, AM fungi could greatly enhance nutrient uptake (Al-Karaki and Al-Radded, 1997; Al-Karaki and Clark, 1999).

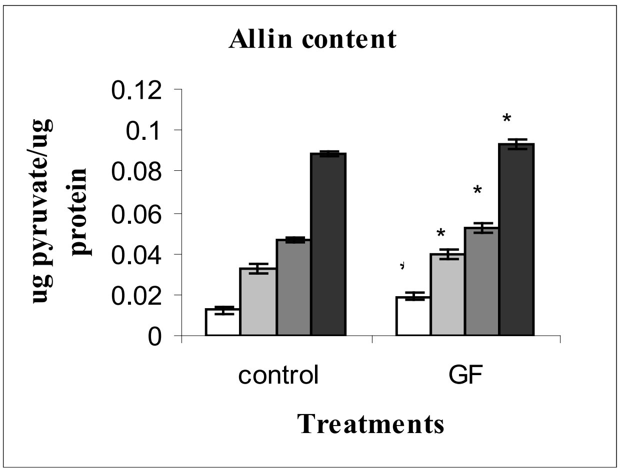

Fig. 2. Allin content in *Allium sativum* L. after 60, 90, 120 and 150 days of AM treatment

Our results also indicate that garlic was dependent on mycorrhiza and responded well to AM inoculant containing intermediate levels of P. Therefore, AM inoculant should be introduced into the soil to ensure satisfactory garlic yield and reduced P fertilization. After harvest, the diameter of bulbs increased significantly in mycorrhizal plants as compared to non mycorrhizal garlic plants (Tab. 2.). The average bulb yield in non mycorrhizal garlic plants was 3.779 ton /ha. and mycorrhizal garlic plants showed 4.575 ton /Ha (Tab. 2.). In mycorrhizal garlic plants, 21.10 % increment in fresh bulb yield was obtained as compared to non mycorrhizal garlic plants (Fig. 4.). The AMF-inoculated garlic plants had higher fresh bulb yields than uninoculated plants grown in field conditions. It may also indicate that the introduced *Glomus fasciculatum* was more efficient than the native fungi, causing an earlier and higher colonization of field-grown garlic. This suggests the

Tab. 2. Yield of non inoculated and non inoculated Allium sativum plant

Treatments	Bulb diameter (cm)	Bulb yield (ton/ha.)
C	7.591±1.064	3.779
GF	7.961±1.082*	4.575*

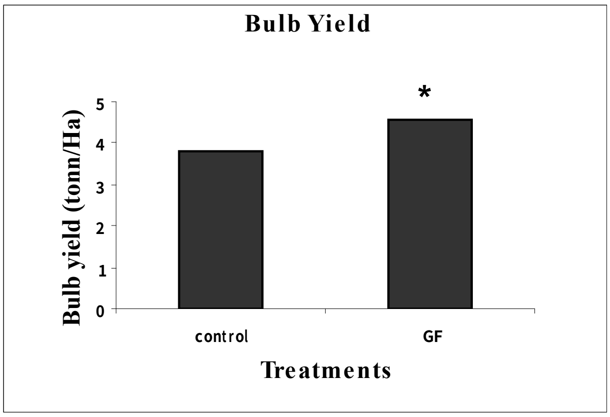

Fig. 4. Bulb yield in *Allium sativum* L. after harvest of AM treatment

potential for supplementing or improving native AM fungi populations to benefit plant growth and hence increase bulb yield.

Conclusions

Our results show that mycorrhizal colonization can contribute substantially to the growth and pungency of garlic plants, and that the effects of mycorrhizal colonization are related to the fungal isolates involved. Whereas suitable fungal species *Glomus fasciculatum* help to increase the yield and to control flavor intensity for consumers. In addition, the promotion of arbuscular mycorrhizal colonization has the advantage of permitting reduced nutrient inputs into the environment.

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